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The usefulness of dermoscopy in canine pattern alopecia: a descriptive study

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Abstract: BACKGROUND: Dermoscopic studies evaluating noninflammatory, nonpruritic progressive alopecia attributable to pattern alopecia are currently unavailable. HYPOTHESIS/OBJECTIVES: To evaluate the dermoscopic features observed in healthy skin of short coated dogs and compare these findings with those observed in dogs affected by pattern alopecia diagnosed by clinical and dermatopathological examination. ANIMALS: Thirty male and female, healthy, breed matched, young adult, short coated dogs (controls) and 30 male and female, young adult, short coated dogs affected by pattern alopecia. METHODS: Dermoscopy was performed with a Fotofinder II videodermoscope equipped with software that allowed the measurement of structures visualized in magnified images (20×-40×-70×). Skin biopsy samples were obtained from the thorax and evaluated dermoscopically for dermoscopic-histological correlation in affected dogs. RESULTS: Dermoscopic findings in canine pattern alopecia were hair shaft thinning, circle hairs and follicular keratin plugs; in the affected sun exposed areas there was a honeycomb-like pattern of pigmentation. Arborizing red lines reflecting vascularization were classified as a nonspecific finding because they were also common in healthy dogs. Dermoscopic features correlated with histology for selected hair follicle abnormalities. CONCLUSIONS AND CLINICAL IMPORTANCE: Although canine pattern alopecia is a visually striking disease, this study supports the value of dermoscopy for clinical examination and also opens promising perspectives for the identification of diagnostic dermoscopic patterns that may be useful for other skin disorders.

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Dermoscopy in dogs: an absorbing perspective in evaluation of pattern alopecia

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Abstract

Background - No dermoscopic studies evaluating non-inflammatory, non-pruritic progressive alopecia attributable to pattern alopecia are currently available.
Hypothesis/objectives - To evaluate the dermoscopic features observed in healthy skin of short-coated dogs and compare these findings with those observed in dogs affected by pattern alopecia diagnosed by clinical and dermatopathologic examination.
Animals - Thirty healthy breed-matched young-adult short-coated dogs, both females and males, were used as controls for the dermoscopic evaluation of 30 young-adult short-coated dogs of both genders affected by pattern alopecia.
Methods - Dermoscopy was performed with the Fotofinder II videodermoscope equipped with software that allowed the measurement of structures visualized in magnified images (20x-40x-70x). Skin biopsy samples were taken at sites evaluated dermoscopically for dermoscopic-histological correlation in affected dogs.
Results - Dermoscopically, canine pattern alopecia was characterized by hair shaft thinning, circle hairs, follicular keratin plugs, and, in the affected sun-exposed areas, by honeycomb-like pattern pigmentation. Arborizing redlines reflecting vascularization were classified as a non-specific finding because they are common also in healthy dogs. Dermoscopic features correlated with histology for selected hair follicle abnormalities.
Conclusions and clinical importance - Although canine pattern alopecia is a visually striking disease, this study supports the value of dermoscopy for clinical

examination and opens promising perspectives for the identification of diagnostic dermoscopic patterns that may be useful for other skin disorders as well.

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Introduction

According to Stolz *et al*, skin surface microscopy dates back to 1663, when Johan Kolhaus first looked at nail fold vessels with a microscope.[1] Nevertheless, it was only at the end of the last century that several diagnostic methods were developed utilizing surface microscopy. Today, the upcoming evidence for *in vivo* diagnosis is represented by dermoscopy originally used to observe and diagnose pigmented skin lesions such as melanocytic nevus and melanoma,[2-4] and trichoscopy as hair and scalp dermoscopy.[5-8] This latter technique has been used to visualize normal hairs and assess their number per follicular unit, to distinguish whether hair follicle openings are normal, empty, fibrotic or containing biological material as hyperkeratotic plugs, and to study the appearance of perifollicular epidermis and cutaneous microvessels.[9] Therefore, trichoscopy has proved relevant in the differentiation of cicatricial from non-cicatricial alopecias. As a large group of disorders characterized by permanent destruction of hair follicles, cicatricial alopecia shows trichoscopic features such as loss of follicular ostia and presence of fibrous tracts that mark extinct follicles.[10] On the other hand, in all non-cicatricial alopecias as alopecia areata and androgenetic alopecia (male and female pattern alopecia), suggestive trichoscopic findings are represented by specific hair shaft and follicular opening abnormalities.[11-13]
Differently from background in humans, to date only a few studies on the application of dermoscopy exist in veterinary medicine and mainly in feline dermatology.[14-16] Moreover, except for an abstract regarding the dermoscopic features of 35 dogs with juvenile-onset demodicosis and 35 breed- and age-matched dogs,[17] the authors are unaware, to the best of their knowledge, of any dermoscopic study on canine non-inflammatory alopecia. Therefore, the purpose of this project was twofold. The first aim was to evaluate dermoscopic features observed in short-coated healthy dogs and compare these findings with those observed in short-coated dogs affected by pattern alopecia diagnosed by clinical and dermatopathological examination. The second aim was to assess whether dermoscopic findings correlated or agreed with those observed at histopathology in order to generate dermoscopic criteria that would be useful for the diagnosis of pattern alopecia.

Material and methods

Study population

A population of 30 healthy short-coated dogs was matched with 30 short-coated dogs referred for non-inflammatory, non-pruritic progressive alopecia attributable to pattern alopecia. Details about both groups are presented in Table 1. Dogs were owned by amateur pet breeders or clients, and informed owner consent was obtained prior to any procedure. Dogs were selected on the basis of the following criteria: (i) no other clinical abnormalities at physical examination; (ii) except for pattern alopecia, no evidence of additional skin lesions on dermatological examination; (iii) for intact female dogs, not being pregnant or lactating; and (iv) normal complete blood count and routine serum biochemical analysis.

Dermoscopic examination

A videodermoscope (Fotofinder® TeachScreen Systems software GmbH Bad Birnbach, Germany) was used and six body sites including convex pinnae, periaural area, ventral neck, thorax, abdomen and caudal thighs were selected. Alcohol (Kodan® spray, Schulke & Mayr, Vienna, Austria) was applied as interface solution to better observe surface and subsurface microscopic features.
In order to take a dermoscopic overview image of the selected cutaneous region, images at 20-fold and 40-fold magnification were first observed. Then, as previously reported by Rakowska *et* al,[11] images at 70-fold magnification, which allows a high-quality enlargement of 9 mm^2 of the skin area to the size of the computer screen, were used for statistical purposes. An area of 3.14 mm^2 was calculated on the selected 70-fold images by means of the FotoFinder® software, and dogs with pattern alopecia and controls were compared for the following parameters: diameter and total number of hair tufts next to follicular ostia per examined area, total number of hairs per hair tuft plus the ratio between the number of secondary hairs/primary hair, and diameter of both primary and secondary hairs in each hair tuft. Hair follicle infundibula, perifollicular epidermis and vascular structures such as very small capillaries were also observed.

Dermoscopy vs. histopathology

To contrast dermoscopy and histopathology, in 20 of the affected dogs a single skin biopsy taken from the thoracic skin area previously circled with a marker during dermoscopic examination was collected under local anaesthesia using a 4-mm skin biopsy punch. The biopsies were fixed in 10% neutral buffered formalin, trimmed, routinely processed, and paraffin embedded. Transverse serial sections (4 µm thick) were obtained and stained with haematoxylin and eosin for histological examination. Histological images were observed under an Olympus BX51 photomicroscope equipped with an Olympus C-5060 Wide Zoom and DP software digital camera (Olympus, Tokyo, Japan) for computer-assisted image acquisition and analysis. The slides contained multiple transverse sections of the skin at different levels starting from the panniculus and ending with the *stratum corneum*. For hair follicle number assessment, transverse skin sections were examined at the level of the mid/lower isthmus. The total number of follicular units per examined area and number of total hairs per follicular unit were counted.
Other parameters included infundibular hyperkeratosis evaluated in the superficial slides at the level of the infundubulus in cross section; vascularization scored in the same slides used to examine infundibular hyperkeratosis; and pigment clumping evaluated in overall sections and scored according to severity of clumping in bulbs and hair shafts. All these findings were graded as - (absent), + (mild), ++ (moderate), +++ (severe).

Statistical analyses

To assess whether dogs with and without pattern alopecia were correctly matched for age and body weight, the Mann-Whitney test was used, and for sex and hair colour the Fisher's exact test and r × c contingency table were used, respectively, within each of the 3 breeds. For each breed investigated, dogs with pattern baldness and controls were compared for the measured parameters on the six body selected regions described above. The analysis was performed using the Mann-Whitney test followed by Bonferroni correction. Furthermore, the same hair parameters were compared between regions within each dog breed for those with and without pattern alopecia, using the Friedman test followed by Dunn's multiple comparison. To assess whether dermoscopic examination yielded similar results to histology, the Spearman's rank correlation coefficient was calculated between the total number of hair tufts next to follicular ostia per examined area based on the former method and the total number of follicular units per examined area counted with the latter. The same test was also used to verify whether the total number of hairs per hair tuft with dermoscopy correlated with the total number of hairs per follicular unit identified with histology. Significance was considered for $P < 0.05$.In addition, the Cohen's kappa coefficient was used to assess whether there was agreement between the two methods in the analysis of infundibular hyperkeratosis, vascularization, and pigment. Kappa values <0 indicated no agreement and 0-0.20 as slight, 0.21-0.40 as fair, 0.41-0.60 as moderate, 0.61-0.80 as substantial, and 0.81-1 as almost perfect agreement. Software package was used for analysis (GraphPad Prism version 5.0, GraphPad Software, La Jolla, CA, USA).

Results

Group matching

Population characteristics did not differ statistically in any of the 3 breeds between dogs with pattern alopecia and controls, suggesting appropriate matching.

Dermoscopic features

On dermoscopy, normal values were considered: hair shafts grouped into follicular units consisting of thick hairs emerging independently from their follicular ostia and considered as primary hairs, and surrounded by a variable number of thinner hairs all protruding through a common external orifice and considered as secondary hairs. Observed were hair follicle openings that were not empty, fibrotic or filled with material such as keratotic plugs; no scaling on perifollicular and interfollicular skin surface; and thin arborizing red lines corresponding to vessels between follicular units. In dogs with dilute hair colour, pinpoint black spots were also observed on interfollicular skin surface.

In dogs affected by pattern alopecia, the most common dermoscopic findings were: hair shaft thinning; scattered circle hairs; plugging of the follicular infundibulum with a yellow-brown material, and on periaural and caudal thigh regions, a honeycomb-like pigmented network. As in controls, pinpoint black spots on interfollicular skin surface of dogs with dilute hair colour and thin arborizing vessels regularly distributed between follicular units were also detected. All these findings are illustrated in Figure 1 (a-f).

Histological findings

In transverse histological sections taken from the thoracic region, hair follicles were characterized by moderate to severe decrease in size (Figure 2a) without distortion or irregularity of their contour or reduction of the overall number of adnexal units (Figure 2b). Infundibular hyperkeratosis and melanin clumping were also variably observed, whereas in some areas, vessels appeared more prominent but were not increased in number.

Dermoscopic parameters in dachshunds

Comparing dachshunds with pattern alopecia and controls, the following significant differences were documented: i) the median diameter of hair tufts next to follicular ostia was smaller in those with pattern alopecia than controls in the convex pinnae (0.05 mm; range 0.03-0.07 *vs* 0.08 mm; range: 0.06-0.09; $P < 0.001$), ventral neck (0.07 mm; range 0.04-0.09 *vs* 0.08 mm; range: 0.07-0.11; $P < 0.01$), chest (0.06 mm; range 0.05-0.08 *vs* 0.08 mm; range: 0.06-0.11; $P < 0.05$) and abdominal region (0.06 mm; range 0.05-0.09 *vs* 0.08 mm; range: 0.06-0.11; $P < 0.01$); and ii) the median diameter of primary hairs was smaller in those with pattern alopecia in the ventral neck (0.03 mm; range 0.02-0.04 *vs* 0.04 mm; range: 0.02-0.05; $P < 0.05$) and chest (0.03 mm; range 0.01-0.04 *vs* 0.04 mm; range: 0.03-0.05; $P < 0.01$). No other differences were documented between groups. In dachshunds with pattern alopecia there was a significant difference in the ratio between the number of secondary hairs/primary hair; in particular, the periaural region had a higher median ratio (7; range: 4-14) than the abdominal region (5; range: 2-8; $P < 0.001$). No other differences were documented for the hair tuft parameters in any region. In controls there were significant differences in the diameter of hair tufts next to follicular ostia and in the diameter of primary hairs; specifically, the periaural region had a smaller median diameter of hair tufts located next to follicular ostia (0.07 mm; range: 0.04-0.08) than the ventral neck (0.08 mm; range: 0.07-0.11; $P < 0.01$), the chest (0.08 mm; range: 0.06-0.11; $P < 0.01$) or abdominal region (0.08 mm; range: 0.06-0.11; $P < 0.05$), while the periaural region had a smaller median diameter of primary hairs (0.03 mm; range: 0.02-0.03) than either the ventral neck (0.04 mm; range: 0.02-0.05; $P < 0.01$) or chest (0.04 mm; range: 0.03-0.05; $P < 0.01$). All these results are summarized in Table 2.

Dermoscopic parameters in Italian greyhounds

Between Italian greyhounds with pattern alopecia and controls, the median diameter of hair tufts next to follicular ostia was smaller in those with pattern alopecia (0.05 mm; range 0.04-0.07) than controls (0.07 mm; range: 0.07-0.08; $P < 0.01$) in the ventral neck. No other differences were documented for the hair tuft parameters in any region. In Italian greyhounds with pattern alopecia there were no significant differences between the 6 regions for any of the 4 hair tuft parameters. Similarly, in controls there were no significant differences. All these results are summarized in Table 2.

Dermoscopic parameters in miniature pinschers

Between miniature pinschers with pattern alopecia and controls, the following significant differences were documented: i) the median diameter of hair tufts next to follicular ostia was smaller in those with pattern alopecia than controls in the convex pinnae (0.05 mm; range 0.05-0.05 *vs* 0.08 mm; range: 0.06-0.10; $P < 0.001$), ventral neck (0.05 mm; range 0.04-0.07 *vs* 0.08 mm; range: 0.07-0.08; $P < 0.05$) and caudal thigh region (0.05 mm; range 0.05-0.06 *vs* 0.07 mm; range: 0.06-0.08; $P < 0.05$); ii) the median diameter of secondary hairs was smaller in those with pattern alopecia than controls in the convex pinnae (0.01 mm; range 0.01-0.01 *vs* 0.02 mm; range: 0.01-0.02; $P < 0.01$), ventral neck (0.01 mm; range 0.01-0.01 *vs* 0.02 mm; range: 0.02-0.02; $P < 0.001$) and chest region (0.01 mm; range 0.01-0.01 *vs* 0.02 mm; range: 0.01-0.02; $P < 0.01$). No other differences were documented between groups. Within pinschers with pattern alopecia there were no significant differences between the 6 regions for any of the 4 hair tuft parameters. In contrast, in controls there were significant differences in the ratio between the number of secondary hairs/primary hair; in particular, the convex pinnae had a higher median ratio (9; range: 8-11) than either the chest (5; range: 5-6; $P < 0.05$) or caudal thigh (5; range: 4-6; $P < 0.01$). All these results are summarized in Table 2.

Dermoscopy vs. histopathology

Dermoscopic and histologic findings are presented in Table 3. A very strong positive correlation was observed for the total number of hair tufts next to follicular ostia based on dermoscopy and the total number of follicular units per examined area counted with histology (rho=0.898; 95% CI=0.750-0.961; $P < 0.001$), and the total number count of hairs per hair tuft at dermoscopy and total number of hairs per follicular unit identified at histology (rho=0.868; 95% CI=0.683-0.948; $P < 0.001$) (Figure 3). A fair agreement was observed between dermoscopy and histology for the analysis of follicular hyperkeratosis (kappa=0.333; 95% CI=0.013-0.679), with only 12 of 20 (60%) agreements; a fair agreement was observed for the analysis of vascularisation (kappa=0.200; 95% CI=0.120-0.520), with only 9 of 20 (45%) agreements; a moderate agreement was observed for the analysis of pigment (kappa=0.294; 95% CI=0.032-0.556), with only 11 of 20 (55%) agreements.

Discussion

In this study, dermoscopic findings in dogs affected by pattern alopecia have been characterized for the first time, highlighting the value of dermoscopy as an adjunctive technique for cutaneous clinical examination.
Canine pattern alopecia is a relatively common but poorly studied skin disorder somehow similar to, but also clearly different from, human androgenetic alopecia.[18] Fine hairs referred to as miniaturized hairs represent the hallmark clinical presentation of this disorder. However, to the best of the authors' knowledge, *in vivo* measurement of hair shaft thickness based on dermoscopy has not been performed before. In this study, the first hair parameter dermoscopically measured was the median hair tuft thickness diameter next to follicular ostia that was shown to be smaller in all affected dogs compared with controls. This result is not surprising if we consider that the relative thinning of hairs is the most striking feature of the disease. Of note, however, differences between breeds and within the same breed were detected, dependent on other hair parameters accounted for. For example, in affected dachshunds the median ratio between the number of secondary

hairs/primary hair was shown to be higher in diseased animals than in controls in all the skin regions evaluated. The periaural region demonstrated the largest number of secondary hairs (7; range: 4-14). Moreover, within the group of dachshund controls the periaural region was demonstrated as having the smallest median diameter of primary hairs (0.03 mm; range: 0.02-0.03) indicating that thinning of hairs in this region may be considered as a normal feature in this breed. In Italian greyhounds, the ventral neck region was described as affected mainly by thinning hairs, and this finding indicates the relevance of this region in distinguishing affected from healthy dogs. In miniature pinschers, secondary hairs were smaller in affected dogs than in controls, mostly in the convex pinnae, ventral neck and chest, whereas in controls, the median ratio between the number of secondary hairs/primary hair was higher in the convex pinnae (9; range: 8-11). All these results taken together reveal that hair shaft thinning in canine pattern alopecia is a process that does not simultaneously affect all hairs of all regions, and that great variability exists between and within affected dog breeds. This variability may be the result of artificial selection pressure for extremely fine haircoats sought by breeders who often attempt to manipulate the appearance of a dog, thereby predisposing it to this presumptively genetic alopecia.[19]

In humans, androgenetic alopecia is considered an inherited condition caused by a genetically determined hair follicle sensitivity to the effects of dihydrotestosterone, with the result of a gradual shortening of anagen phase and a prolongation of kenogen phase.[20-24] Increased concentrations of both 5-α reductase isoenzyme and androgen receptor have been detected in the balding scalp, suggesting that such changes contribute to hair loss.[25]

To date, the pathogenesis of canine pattern alopecia is not known and the involvement of an abnormality in hair follicle hormonal receptor is still debated.[19] The alteration of the hair-cycle dynamics with an increase in the prevalence of kenogen follicles has been demonstrated in some canine non-inflammatory alopecias but not in pattern alopecia,[26] and the expression of 5-α-reductase genes has been evaluated in only one study, performed in normal skin.[27]

In order to provide both qualitative and quantitative diagnostic follicular information, transverse sections of skin biopsy specimens were used in this study, as in human literature.[28,29] Some key information such as follicular counts was easily assessed, and histological findings were shown to positively correlate with dermoscopic calculations of hair parameters. However, accurate determination of growth stages of the hair cycle was not possible on transverse sections due to the absence of the entire length of the hair follicle including site, shape and depth of the hair inferior portion and, specifically, of the bulb. Therefore longitudinal sections continue to provide the best morphological and spatial information to assess specific growth stages of hair cycle in dogs.[26] Additionally, in human beings hairs are mostly primary while in normal and diseased skin of dogs it is difficult to determine the primary or secondary origin of the follicle, especially without the hair shaft.

To detect other dermoscopic features that could differentiate diseased dogs from controls, hair follicle openings, perifollicular and interfollicular skin surface, and vascular structures were dermoscopically examined and evaluated in conjunction with histological findings. Follicular ostia filled with light yellowish or brownish material were mostly observed in the ventral regions of dogs affected by pattern alopecia; this was histologically related to a variable amount of keratin filling the follicular infudibulum. In humans, this dermoscopic finding, termed ‘yellow dot’, represents sebum mixed with variable amounts of keratin secreted by normal, active sebaceous glands through the miniaturized hair follicle.[7,9,11] Therefore the result of

this process is the accumulation of yellow material at the top of the hair follicular opening. Our hypothesis is that a similar mechanism may occur in canine pattern alopecia.
Moreover, in some affected dogs, hairs with typical circular or spiraliform arrangement were dermoscopically observed, but no histological change was identified in relation to this dermoscopic feature. In humans, the pathogenesis of this finding remains obscure, although some authors relate it to hairs with a small diameter that renders it difficult to penetrate the *stratum corneum*. For this reason, they grow in a circular tract and in a subcorneal location.[30] Based on this, our dermoscopic finding may have an explanation, but further studies are needed to better understand the pathogenesis of these hairs with this typical arrangement.
Variable infundibular melanin clumping in both healthy and affected dogs with dilute hair colour was histologically detected and dermoscopically visualized as pinpoint black spots on interfollicular skin surface.
Finally, a honeycomb-like hyperpigmentation pattern, characterized by hyperchromic rings on the skin surface and resulting from solar exposure in thinning or completely balding areas as demonstrated in humans,[10] often coexisted as an additional feature in the periaural and caudal thigh regions.
Cutaneous microvessels that arborize into thin red branches in a non-homogeneous fashion were considered as non-specific dermoscopic findings because they are also common in normal skin. Given that dermoscopy enables horizontal inspection of the skin, vessels that run parallel to the skin surface are visualized as lines, while those that run perpendicularly are generally viewed as dots, or even loops.[31,32] However, they are best evaluated when the pressure exerted by the dermoscope against the skin is low. High outside pressure may indeed reduce blood flow in cutaneous capillaries.[10] In this study, the lack of dermoscopic visualization of cutaneous blood vessels in some selected areas may have resulted from excessive pressure applied to the skin. Translucent ultrasound gel that allows one to apply the lens against the skin gently in order to better visualize blood vessels is expected in future studies.
In summary, the results of this study suggest that dermoscopy may provide a new, relevant clinical perspective on hair disorders and offer the clinician a novel way in which to uncover clinical aspects of cutaneous diseases.

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Figure legend

Figure 1. Representative dermoscopic features in dogs affected by pattern alopecia. (a) Diffuse hair thinning (20x). (b) Presence of hair circle (black arrows) between miniaturized hairs (20x). (c) Hair circles (black arrow), thin arborizing vessels (red arrow) and yellow-brown material around follicular ostia (blue arrow) (20x). (d) Plugging of follicular infundibular with yellow-brown material (blue arrows) (70x). Pinpoint black dots in a dog with diluted hair colour (black arrows) (70x). Honeycomb-like pattern (black arrows) on the caudal thigh (70x).

Figure 2. Representative photomicrographs of hair follicle miniaturization in dogs affected by pattern alopecia. (a) Decrease in size of hair follicular units without distortion or irregularity of their contour or reduction of the overall numbers of adnexal units. Scale bar represents 1000 µm. (b) Multiple thinner hair follicles at higher magnification. Scale bar represent 200 µm.

Figure 3. Correlation of the number of hair tufts located next to follicular ostia based on dermoscopy (x-axis) with number of follicular units counted with histology (y-axis). The regression line is shown.